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ABSTRACT

This document is an instructional module package prepared in objective form for use by an instructor familiar with the azide modification of the Winkler dissolved oxygen test and the electronic dissolved oxygen meter test procedures for determining the dissolved oxygen and the biochemical oxygen demand of a wastewater sample. Included are objectives, instructor guides, student handouts, and transparency masters. This module considers sampling, test procedures and graduated cylinder and direct dilution methods. (Author/RH)

ED152582

BIOCHEMICAL OXYGEN DEMAND AND DISSOLVED OXYGEN

Training Module 5.105.2.77

Prepared for the

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by

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September, 1977

The mention of trade names, or use of manufacturers technical bulletins, diagrams depicting specific equipment, or the commercial product in this module is for illustration purposes, and does not constitute endorsement or recommendation for use by Kirkwood Community College nor by the Iowa Department of Environmental Quality.

Module No:	Module Title:
Approx. Time:	Topics: Introduction and Sampling Winkler DO Test BOD Tests DO Meters

Objectives:

When the participants complete this topic they should be able to:

Determine the dissolved oxygen content in water sample.

Determine the biochemical oxygen demand of a water sample.

Instructional Aids:

Handouts
EPA video tapes

Instructional Approach:

Lecture
Lab

References:

Standard Methods
EPA Effluent Monitoring Procedures

Class Assignments:

Instructional Aids

EPA slide-tape is available from:

Eileen Hopewell
National Training Center
Water Programs Operation
Vine & St. Claire St.
Cincinnati, Ohio 45268

Overheads

Typed overheads are an example of overhead layout and content. For classroom use the overhead should be constructed using colored, 1/4 inch dry transfer letters.

Other overheads may be copied directly.

Handouts

Handouts may be copied directly.

Lab supplies and apparatus

Supplies and apparatus should be supplied per handouts so that participants may work in groups of 2 or 3.

Module No:	Module Title: DO & BOD Testing
	Submodule Title: Winkler DO Test
Approx. Time: 1 hour	Topic: Introduction and Sampling

Objectives:

When the participants complete this topic they should be able to:

1. Indicate the relationship between dissolved oxygen and temperature.
2. Determine the saturation value of dissolved oxygen in water given water temperature, barometric pressure and saturation values table.
3. Identify the proper apparatus and reagents needed for taking a dissolved oxygen sample for the Winkler DO method.
4. Obtain and prepare a proper dissolved oxygen sample for the Winkler method.

Instructional Aids:

Handout
Lab equipment

Instructional Approach:

Lecture
Lab

References:

Standard Methods
EPA, EMP

Class Assignments:

Module No:	Topic: Introduction and Sampling

Instructor Notes:	Instructor Outline:
	1. Introduce the relationship between a. DO and temperature b. Give examples of how this relationship may affect results of DO tests
Handout - Page 7	2. Demonstrate the use of DO saturation table
Handout - Page 15	3. a. Discuss the use of the DO sampling equipment APHA Sampler Kemmerier samples Direct sampling Bottle sampler b. Discuss sample preservation c. Discuss the chemistry of the reaction
	4. a. Demonstrate proper sampling method and preservation of a DO sample for a Winkler test. b. Have class preserve a DO sample

DISSOLVED OXYGEN

Temperature		Pressure in Millimeters and Inches Hg			
		775	760	750	725
F°	C°	30.51	29.92	29.53	28.54
32.0	0	14.9	14.6	14.4	13.9
33.8	1	14.5	14.2	14.1	13.6
35.6	2	14.1	13.9	13.7	13.2
37.4	3	13.8	13.5	13.3	12.9
39.2	4	13.4	13.2	13.0	12.5
41.0	5	13.1	12.8	12.6	12.2
42.8	6	12.7	12.5	12.3	11.9
44.6	7	12.4	12.2	12.0	11.6
46.4	8	12.1	11.9	11.7	11.3
48.2	9	11.8	11.6	11.5	11.1
50.0	10	11.6	11.3	11.2	10.8
51.8	11	11.3	11.1	10.9	10.6
53.6	12	11.1	10.8	10.7	10.3
55.4	13	10.8	10.6	10.5	10.1
57.2	14	10.6	10.4	10.2	9.9
59.0	15	10.4	10.2	10.0	9.7
60.8	16	10.1	9.9	9.8	9.5
62.8	17	9.9	9.7	9.6	9.3
64.4	18	9.7	9.5	9.4	9.1
66.2	19	9.5	9.3	9.2	8.9
68.0	20	9.3	9.2	9.1	8.7
69.8	21	9.2	9.0	8.9	8.6
71.6	22	9.0	8.8	8.7	8.4
73.4	23	8.8	8.7	8.5	8.2
75.2	24	8.7	8.5	8.4	8.1
77.0	25	8.5	8.4	8.3	8.0
78.8	26	8.4	8.2	8.1	7.8
80.6	27	8.2	8.1	8.0	7.7
82.4	28	8.1	7.9	7.8	7.6
84.2	29	7.9	7.8	7.7	7.4
86.0	30	7.8	7.7	7.6	7.3
87.6	31	7.7	7.5	7.4	7.2

Module No:	Module Title:
	Submodule Title: Winkler DO Test
Approx. Time: 2 hours	Topic: DO Test

Objectives:

When the participants complete this topic they should be able to:

1. Identify the proper apparatus and reagents needed for the azide modification of the Winkler DO test.
2. Conduct a dissolved oxygen test using the azide modification of the Winkler method given test equipment, procedures sheet and proper sample material.
3. Translate the raw data from the DO test into proper units of expression.

Instructional Aids:

Handout
Lab equipment
EPA video tape

Instructional Approach:

Lab

References:

Standard Methods

Class Assignments:

Module No:	Topic: DO Test

Instructor Notes:	Instructor Outline:
EPA Video Tape Handout - Pages 10 - 17	1. a. Identify the proper apparatus and reagents needed for the Winkler DO test. b. Demonstrate the makeup of reagents and indicate that they may be bought pre-made. c. Discuss the use of alternate reagents PAO. 2. a. Demonstrate the test procedure to the class. b. Have class conduct the test.
Page 17	3. a. Work example problems. b. Have class convert their raw data into proper units of expression.

Winkler Determination of Dissolved Oxygen-Azide Modification

1. Analysis Objectives:

   The operator will be able to perform a Winkler dissolved oxygen determination, using the azide modification, on a sewage sample.

2. Brief Description of Analysis:

   A solution of manganous sulfate is added to the sample. A solution containing sodium hydroxide, sodium iodide and sodium azide is next added. If oxygen is present in the sample, a brown flocculent precipitate forms. If no oxygen is present, a white precipitate forms. Sulfuric acid is then added to the sample, and the precipitate dissolves. The solution is titrated with P.A.O. using starch indicator. At the end point of the titration, the color of the solution changes from pale blue to colorless. The milliliters of P.A.O. used, is equal to the milligrams of dissolved oxygen per liter of sample.

General Description of Equipment Used in the Process

A. Capital Equipment

   1. Analytical balance, 200 g. capacity
   2. Trip balance, 500 g. capacity
   3. Oven, temperature controllable to ± 2° C, large enough to hold a small evaporating dish
   4. Refrigerator, large enough to hold three 1 liter bottles
   5. Still, or other source of distilled water

B. Reusable

   1. Hot plate, large enough to hold a 2 liter Erlenmeyer flask
   2. Kemmerer sampler

3. APHA sampler
4. Laboratory apron
5. Safety glasses
6. Brushes (for cleaning glassware)
7. Brush (for cleaning balance)
8. One 1 liter volumetric flask
9. One 300 ml BOD bottle
10. One 1 liter graduated cylinder
11. One 100 ml graduated cylinder
12. One 50 ml graduated cylinder
13. One 10 ml graduated cylinder
14. Six 1 liter glass stoppered bottles
15. One rubber stopper (to fit a 1 liter glass stoppered bottle)
16. One 150 ml glass stoppered bottle
17. One spatula (medium size)
18. One spatula (small size)
19. One 2 liter Erlenmeyer flask
20. One 500 ml wide mouth Erlenmeyer flask
21. One 100 ml pipet
22. One 50 ml pipet
23. One 20 ml pipet
24. One pipet bulb
25. Three 5 ml graduated pipets
26. One desiccator (large enough to hold a small evaporating dish)
27. One evaporating dish (large enough to hold about 10 g. of solid)

28. One 25 ml buret

29. One ring stand

30. One buret clamp

31. One distilled water plastic squeeze bottle

32. One pen or pencil

33. One notebook (for recording data)

34. Eight plastic weighing boats (2-3 inches square)

35. Sponges (for cleaning of laboratory table tops)

36. One stirring rod (about 6 inches long)

37. One powder funnel, about 3 inch diameter.

C. Consumable

1. Potassium dichromate, $K_2Cr_2O_7$

2. Concentrated sulfuric acid, H_2SO_4

3. Soap

   (These three reagents are for cleaning glassware. The quantities needed will therefore vary.)

4. 480 g. sodium hydroxide, NaOH

   400 g. manganous sulfate dihydrate, $MnSO_4.2H_2O$, or 364 g. manganous sulfate monohydrate, $MnSO_4.H_2O$ may also be used.

5. 500 g. sodium hydroxide, NaOH

6. 135 g. sodium iodide, NaI

7. 10 g. sodium azide, NaN_3

8. [illegible] g. soluble starch

9. 15 ml. chloroform, $CHCl_3$

10. Sodium dichromate, $Na_2Cr_2O_7$

11. 20 ml (P.A.O.) Phenylarsine Oxide 0.0375 N

The quantities tiven in 4 through 9 above will suffice for approximately 450 determinations of dissolved oxygen. Depending on usage, smaller quantities may be prepared.

All reagents should be of high quality. Different chemical manufacturers may have different ways of indicating a high quality reagent. While no endorsement of one chemical manufacturer over another is intended, the following are some designations used in four chemical catalogs to indicate high quality reagents.

Catalog	Designations
Thomas	Reagent, ACS, Chemically Pure (CP)
Matheson, Coleman & Bell	Reagent, ACS
Curtin Matheson Scientific Inc.	Primary Standard, ACS, AR
Fisher	Certified, ACS

A. Equipment Preparation

1. Cleaning of glassware

   a. Clean all glassware and rinse with distilled water.

2. Balance preparation

   a. Check all balances for cleanliness and proper operation

B. Reagent Preparation

1. Manganous sulfate solution

   a. Prepare 1 liter of solution containing 480 g. of manganous sulfate tetratrydrate, $MnSO_4.4H_2O$.

      1. Unless otherwise specified, solutions should be stored in glass stoppered bottles.

      2. Unless otherwise specified, the term water means distilled water.

2. Alkaline iodide azide solution

a. Dissolve 500 g. of sodium hydroxide, NaOH, in 500 ml of water.

1. Caution: Heat is generated.

b. Cool the solution to room temperature.

c. Dissolve 135 g. of sodium iodide, NaI, in 200 ml of water.

d. Dissolve 10 g. of sodium azide, NaN_3, in 40 ml of water.

e. Combine the three solutions and dilute to 1 liter.

1. This solution should be stored in a glass bottle fitted with a rubber stopper, or in a clean plastic bottle.

3. Starch solution

a. Gently boil 1 liter of water on a hot plate.

1. Proceed with the next steps while the water is heating and boiling.

b. Weigh 10 g. of soluble starch.

c. Transfer it to a mortar.

d. Add about 3 ml of water.

e. Grind with a pestle so as to form a thin paste.

f. Pour the paste into the boiling water.

g. Allow the solution to stand overnight.

h. Decant the starch solution into a bottle.

1. Decanting means to pour slowly so that any solid material will be left behind.

i. Add 5 ml of chloroform, $CHCl_3$.

1. Store in a refrigerator

C. Determination of Dissolved Oxygen

a. Sample collection

1. If the sample is to be collected from a depth greater than 5 feet, use a Kemmerer sampler.
2. If the sample is to be collected from a depth less than 5 feet, use an APHA sampler containing a 300 ml BOD bottle.
3. If a Kemmerer is used, transfer the sample to a 300 ml BOD bottle. Allow some of the sample to overflow.
   a. Caution: During the sample transfer, do not allow it to splash.
4. Carefully insert the stopper of the BOD bottle.
   a. Do not create any air bubbles in the bottle.
5. For surface samples, the sample may be collected directly in a 300 ml BOD bottle.
   a. Fill the bottle in such a way that no turbulence is created.

b. Addition of reagents

1. Remove the stopper and pipette 2.0 ml of manganous sulfate solution into the sample.
   a. Have the tip of the pipette about 1/2 inch below the surface of the liquid. It is desirable, but not necessary, that the normality be 0.0375.
2. Pipette 2.0 ml of alkaline iodide azide solution into the sample, over the sink.
   a. Have the tip of the pipette about 1/2 inch below the surface of the liquid.
   b. A precipitate forms.

3. Carefully insert the stopper of the BOD bottle.
   a. Do not create any air bubbles in the bottle.
4. Rinse off the outside of the BOD bottle.
   a. The alkaline iodide azide solution is damaging to the skin.
5. Holding the hand over the stopper, invert the BOD bottle slowly 5 times.
6. Allow the precipitate to settle.
   a. If it does not settle, wait 2 minutes and proceed.
7. Repeat the shaking and settling steps.
8. Pipette 2.0 ml of concentrated sulfuric acid into the sample.
   a. The pipette need not be below the surface of the liquid.
9. Carefully insert the stopper of the BOD bottle.
   a. Do not create any air bubbles in bottle during this step.
10. Rinse off the outside of the BOD bottle.
11. Holding the hand over the stopper, invert the BOD bottle slowly five times.
    a. The precipitate will dissolve.
    b. The color of the solution is red-brown if oxygen is present, but colorless if no oxygen is present. If the solution is yellow, a small amount of oxygen is present.

c. Titration

1. Transfer the entire contents of the 300 ml BOD bottle to a wide mouth 500 ml Erlenmeyer flask.
2. Add the P.A.O. titrant from a buret until the red-brown color changes to a pale yellow

a. If there was little oxygen in the sample and the yellow color was therefore present even before addition of any P.A.O., the starch should be added immediately.

3. Add 2 ml of starch solution

   a. A medium blue-pale blue color will form.

4. Continue the titration until the color changes from pale blue to colorless.

   a. Ignore any return of blue color.

5. Record the ml P.A.O. used.

d. Calculations

1. Calculate the mg of DO per liter of sample.

   a. mg DO/liter = ml of P.A.O. titrant X N of P.A.O. titrant X 8 X 1000/ml of sample

   b. Since the sample was in a 300 ml BOD bottle, mg DO/liter = ml of P.A.O. X N of P.A.O. X 8 x 1000/300

   c. Or mg DO/liter = ml of P.A.O. X N of P.A.O. X 26.7

   d. If the N of the P.A.O. was exactly 0.0375, then mg DO/liter = ml of P.A.O. X 0.0375 X 8 X 1000/300.

   e. Or mg DO/liter = ml of P.A.O. X 1

Module No:	Module Title:
	Submodule Title: BOD
Approx. Time: 1 hour	Topic: Introduction

Objectives:

When the participants complete this topic they should be able to:

1. Indicate what the BOD test measures and what is the limiting factor in the test.
2. Indicate three types of wastewater that a BOD test may not give valid results.
3. Explain seeding of BOD samples and indicate what types of wastewater must be seeded.
4. Indicate the proper preservative and holding time limit for a BOD sample.
5. Indicate the proper sample dilution given the estimated BOD of the sample.

Instructional Aids:

Handout

Instructional Approach:

Lecture

References:

Standard Methods

Class Assignments:

Module No:	Topic: Introduction

Instructor Notes:	Instructor Outline:
	1. a. Discuss what the BOD test measures. b. Discuss how it is measured. c. Indicate why it is the standard test rather than COD or TOD. 2. Indicate types of samples that will not give proper BOD results and why they will not give proper results. 3. Discuss sample seeding. 4. Discuss sampling and holding time. 5. a. Discuss sample dilution. b. Work example problems. c. Have class calculate dilutions required given an estimated BOD.

Module No:	Module Title:
	Submodule Title: BOD
Approx. Time:	Topic: Graduated Cylinder Dilution Method

Objectives:

When the participants complete this topic they should be able to:

1. Identify the proper apparatus and chemicals needed to set up BODs by the graduated cylinder dilution method.
2. Conduct a BOD test (graduated cylinder dilution method) given proper test equipment, procedures sheet, and sample.
3. Translate the raw data from the BOD test into proper units of expression given appropriate equations.

Instructional Aids:

Handout
EPA video tape
Lab equipment

Instructional Approach:

Lab

References:

Standard Methods
EPA EMPs Manual

Class Assignments:

Module No:	Topic: Graduated Cylinder Dilution Method

Instructor Notes:	Instructor Outline:
Pages 22 - 31	1. a. List and identify the proper apparatus needed for BOD test.
	b. List and identify chemicals and reagents needed.
	c. Indicate that reagents may be bought pre-made.
	d. Discuss care of buffer reagent.
(To the point of incubation)	2. a. Demonstrate set up of BOD test.
	b. Have participants set up a BOD test.
	3. a. Demonstrate calculations using data from a finished BOD test.
	b. Have participant work calculations using data from a finished test.

Effluent Monitoring Procedure: Determination of Five-day Biochemical Oxygen Demand (BOD_5)

1. Analysis Objectives:

   The learner will determine the five-day biochemical oxygen demand of a sewage sample.

2. Brief Description of Analysis:

   The sample is diluted with a high quality distilled water containing nutrient salts and a buffer. Two biochemical oxygen demand (BOD) bottles are filled with the diluted sample. The dissolved oxygen (DO) content of the first bottle is determined, and expressed as mg of DO/liter. The second bottle is stored in the dark at 20° C. for five days. During the five-day period, microorganisms in the sample break down complex organic matter in the sample, using up oxygen in the process. At the end of the five-day period, the DO content of the second BOD bottle is determined, and again expressed as mg of DO/liter. The depletion in oxygen content, divided by the percent of sample used (expressed as a decimal fraction) is the five-day biochemical oxygen demand expressed as milligrams of BOD per liter of sample. BOD_5 is the symbol for the five-day biochemical oxygen demand.

General Description of Equipment Used in the Process

A. Capital

   1. Trip balance, 100 g capacity
   2. Still, or other source of distilled water
   3. Incubator capable of maintaining a temperature of 20° C. ± 1° C, and large enough to hold four 300 ml BOD bottles and a 3 liter jug or bottle.

B. Reusable

1. Brushes (for cleaning glassware)
2. Brush (for cleaning balance)
3. Laboratory apron
4. Safety glasses
5. One spatula (medium size)
6. One distilled water plastic squeeze bottle
7. One pen or pencil
8. One notebook (for recording data)
9. Seven plastic weighing boats (2-3 inches square)
10. Sponges (for cleaning of laboratory table tops)
11. One 3 liter jug or bottle with narrow neck
12. One powder funnel, about 3 inch diameter
13. One 1 liter volumetric flask
14. Four 1 liter glass stoppered bottles
15. Two 1 liter graduated cylinders
16. One siphon (long enough for use with the 1 liter graduated cylinder)
17. Four 1 ml volumetric pipets
18. One 10 ml volumetric pipet
19. One 20 ml volumetric pipet
20. One plunger type mixer (for use with the 1 liter graduated cylinder
21. Four 300 ml BOD bottles
22. Equipment for doing a Winkler DO determination-azide modification, see EMP CH.O.EMP.1.8.74, Determination of dissolved Oxygen-Azide Modification, or

23. One dissolved oxygen meter, see EMP CH.O.DO. EMP. 1.8.74, Determination of dissolved Oxygen Using a Dissolved Oxygen Meter.
24. One 2 liter beaker (for preparing cleaning solution)
25. One 12 inch stirring rod (for preparing cleaning solution).

C. Consumable

1. Small wad of cotton (to plug the 3 liter jug or bottle)
2. 8.5 g. of potassium dihydrogen phosphate, KH_2PO_4
3. 21.75 g. of dipotassium hydrogen phosphate, K_2HPO_4
4. 33.4 g. of disodium hydrogen phosphate heptahydrate, $Na_2HOP_4.7H_2O$
5. 1.7 g of ammonium chloride, NH_4Cl
6. 22.5 g. of magnesium sulfate heptahydrate, $MgSO_4.7H_2O$
7. 27.5 g. of anhydrous calcium chloride, $CaCl_2$
8. 0.25 g. of ferric chloride hexahydrate, $FeCl_3.6H_2O$
9. Reagents for doing a Winkler DO determination-azide modification, see EMP CH.O.EMP.1a.9.74, Winkler Determination of Dissolved Oxygen-Azide Modification.
10. Reagents for use with a dissolved oxygen meter, see EMP CH.O.do.EMP. 1a.9.74, Determination of Dissolved Oxygen Using a Dissolved Oxygen Meter.
11. Concentrated sulfuric acid, H_2SO_4
12. Sodium Dichromate, $Na_2Cr_2O_7$
13. Soap

(Items 11, 12, and 13 are for cleaning glassware. The quantities needed will therefore vary).

A. Equipment Preparation

1. Cleaning of glassware

a. Clean all glassware and rinse with distilled water.

2. Balance inspection

a. Check all balances for cleanliness and proper operation.

B. Reagent Preparation

1. Distilled water

a. Distill 2 liters of water into a small neck jug (or large bottle).

1. Unless otherwise specified, the term water means distilled water.

b. Plug the jug with a loose fitting piece of cotton.

1. Unless otherwise specified, solutions should be stored in glass stoppered bottles.

c. Store the jug at 20° C ± 1° C. for 48 hours prior to use.

1. This length of time has been determined simply on the basis of experience.

d. Or aerate the water just prior to use.

1. Do this by shaking the water in a half-filled jug, or

2. By using a clean supply of compressed air. (Be cautious about air jets and motors which may simply contaminate the water with oil).

2. Phosphate buffer solution

a. Weigh ~~8.5~~ g. of potassium dihydrogen phosphate, KH_2PO_4

b. Weigh 21.75 g. of dipotassium hydrogen phosphate, K_2HPO_4.

c. Weigh 33.4 g. of disodium hydrogen phosphate heptahydrate, $Na_2HPO_4.7H_2O$

d. Weigh 1.7 g. of ammonium chloride, NH_4Cl

e. Dissolve the four chemicals together in about 500 ml of water.

f. Dilute to 1 liter.

3. Magnesium sulfate solution

a. Dissolve 22.5 g. of magnesium sulfate heptahydrate, $MgSO_4.7H_2O$, in water and dilute to 1 liter.

4. Calcium chloride solution

a. Dissolve 27.5 g. of anhydrous calcium chloride, $CaCl_2$, in water and dilute to 1 liter.

5. Ferric chloride solution

a. Dissolve 0.25 g. of ferric chloride, $FeCl_3$, in water and dilute to 1 liter.

C. Procedure

1. Sample dilution

a. Siphon about 500 ml of the 20° C. water into a 1 liter graduated cylinder.

1. Do not cause splashing which might change the oxygen content of the water.

b. Add 1.0 ml of each of the magnesium sulfate, calcium chloride, ferric chloride, and phosphate buffer solutions.

1. Mix gently with a plunger-type mixer after adding each of the four solutions. Cause no splashing.

2. One ml of each of the 4 solutions is used for each liter of water.

3. These 4 solutions may be added to the reservoir of 20° C. water just prior to use.

c. Shake the sample container, measure the sample, and add it to the graduated cylinder.

1. For influents of domestic wastewaters:

   10.0 ml (1% of the liter volume)

   0.0 ml (2% of the liter volume)

   40.0 ml (4% of the liter volume)

   For effluents from primary treatment plants:

   40.0 ml (4% of the liter volume)

   60 ml (6% of the liter volume)

   80 ml (8% of the liter volume)

   For effluents from secondary treatment plants:

   200 ml (20% of the liter volume)

   300 ml (30% of the liter volume)

   400 ml (40% of the liter volume)

2. The sample volumes above are suggested values. The actual sample volume for each kind of waste must be determined by experience.

d. Siphon in additional 20° C water to the 1 liter mark.

1. Do not cause splashing.

2. Other sample dilution methods are sometimes used; e.g., a 1 liter volumetric flask in place of the graduated cylinder.

e. Use a plunger-type mixer to mix the contents of the cylinder.

1. Mix gently so as not to cause splashing.

D. BOD bottle filling

1. For each sample volume used, fill 2 BOD bottles by siphoning from the liter cylinder.
   a. Hold the end of the siphon near the bottom of the BOD bottle so as to prevent splashing.
   b. Open the siphon slowly.
2. Stopper the BOD bottles.
   a. Do not cause formation of an air bubble by inserting the stopper too vigorously.

E. Blank determination

1. Siphon about 500 ml of the 20° C. water into a 2nd one liter graduated cylinder.
   a. Cause no splashing.
2. Add 1.0 ml each of the magnesium sulfate, calcium chloride, ferric chloride, and buffer solutions.
   a. Mix gently with a plunger-type mixer after adding each of the four solutions. Cause no splashing.
3. Siphon in additional 20° C. water to the 1 liter mark.
   a. Cause no splashing.
   b. No sample is used in this second cylinder.
4. Mix the contents of this second cylinder using a plunger-type mixer.
5. By siphoning, fill 2 BOD bottles with this mixture.
   a. These two bottles are called blanks.

F. DO determination

1. Fill the flared top of one of the sample and one of the blank BOD bottles with water.

2. Store them at 20° C. in the dark for 5 days.

   a. Check the flared tops daily and refill with water if necessary.

   b. There are alternate ways of storing the bottles at 20° C. so as to maintain a water seal.

3. Determine the DO of the 2nd sample and blank bottles.

   a. Use the Winkler method-azide modification, or a dissolved oxygen meter.

   b. This determination should be done within 15 minutes after filling the BOD bottles.

   c. These DO values are often called initial DO values.

4. After 5 days, determine the oxygen content of the stored sample and blank BOD bottles.

   a. Use the same method as before.

G. Calculations

1. Subtract the DO (expressed in mg/l) value of the fifth day sample bottle from the initial DO (expressed in mg/l) value of the first bottle.

   a. e.g. 5.0 = mg initial DO in the first bottle, and 2.0 = mg DO in the stored bottle after 5 days.

   b. 5.0 - 2.0 = 3.0

2. Divide the difference by the percent of sample used, expressed as a decimal; the answer is mg BOD_5/l.

   a. e.g., if 10% sample was used, then mg BOD_5/liter = 3.0/0.1 = 30.

   b. If the decrease in DO over the 5 day period is not at least 2.0 mg/liter, the BOD_5 result will not be reliable and should be ignored.

c. If there is not at least 1 mg of DO left in the stored bottle after 5 days, the BOD_5 result will not be reliable and should be ignored.

d. It is common to set up at least 3 dilutions of a particular sample so that 1 of the bottles will show an acceptable oxygen depletion over the 5 day period.

3. For the blank BOD bottles, subtract the ml of sodium thiosulfate titrant used for the stored bottle from the ml used for the initial bottle.

   a. The difference should not be greater than 0.2 ml.

   b. If it is, the 20° C. water is of low quality.

   c. Possible causes are organic contamination in the water (check the aeration procedure) or dirty glassware (especially the BOD bottles and water storage jug) which has contaminated the water.

   d. The difference in ml readings is not used as a blank correction, but merely as a check on the quality of the 20° C. water.

   e. One example of a data sheet is attached.

B. O. D. WORKSHEET

SAMPLE SOURCE ________ DATE ________ TIME ________
DILUTION ________ SAMPLE VOLUME ________ DILUTED TO ________ MILLITERS
BOTTLE # ________ 15 MIN. DO ________ AVERAGE 5 DAY DO ________
BOTTLE # ________ 5 DAY DO ________ CHANGE IN DO ________
BOTTLE # ________ 5 DAY DO ________ (15 MIN. DO - AVE. 5 DAY DO)
DATE IN ________ DATE OUT ________ 5 DAY BOD

- -

SAMPLE SOURCE ________ DATE ________ TIME ________
DILUTION ________ SAMPLE VOLUME ________ DILUTED TO ________ MILLITERS
BOTTLE # ________ 15 MIN. DO ________ AVERAGE 5 DAY DO ________
BOTTLE # ________ 5 DAY DO ________ CHANGE IN DO ________
BOTTLE # ________ 5 DAY DO ________ (15 MIN. DO - AVE. 5 DAY DO)
DATE IN ________ DATE OUT ________ 5 DAY BOD

- -

SAMPLE SOURCE ________ DATE ________ TIME ________
DILUTION ________ SAMPLE VOLUME ________ DILUTED TO ________ MILLITERS
BOTTLE # ________ 15 MIN. DO ________ AVERAGE 5 DAY DO ________
BOTTLE # ________ 5 DAY DO ________ CHANGE IN DO ________
BOTTLE # ________ 5 DAY DO ________ (15 MIN. DO - AVE. 5 DAY DO)
DATE IN ________ DATE OUT ________ 5 DAY BOD

- -

BLANK DETERMINATION:

BOTTLE # ________ 15 MIN. DO ________ BOTTLE # ________ 15 MIN DO ________
BOTTLE # ________ 5 DAY DO ________ BOTTLE # ________ 5 DAY DO ________
AVERAGE 15 MIN. DO ________ AVERAGE 5 DAY DO ________
CHANGE IN DO ________
(AVE. 15 MIN. DO - AVE. 5 DAY DO)

- -

$$5 \text{ DAY BOD} = \frac{100 \times \text{CHANGE IN DO}}{\% \text{ DILUTION}}$$

Module No:	Module Title:
	Submodule Title:
Approx. Time: 2 hours	Topic: DO Meters

Objectives:

When the participants complete this topic they should be able to:

1. Identify the component parts of a DO meter and probe.
2. Prepare the DO probe for operation by changing the membrane and adding new electrolite.
3. Standardize the DO meter.
4. Conduct a DO test using the DO meter.

Instructional Aids:

Lab equipment

Instructional Approach:

Lecture
Lab

References:

DO Meter instructions
Standard Methods, 14th Edition

Class Assignments:

Module No.	Topic: DO Meters

Instructor Notes:	Instructor Outline:
	1. a. Identify the parts of a DO Meter and probe.
	b. Indicate the chemistry of the reaction.
	2. a. Demonstrate how to change the membrane on the DO probe.
	b. Have participants change the membrane.
DO Meter Instructions	3. a. Demonstrate how to standardize the meter.
	b. Discuss other methods of standardization.
	c. Have participants standardize their meters.
	4. a. Demonstrate the DO test using a meter.
	b. Have participants run a DO test.

Module No:	Module Title:
	Submodule Title: BOD
Approx. Time: 2 hours	Topic: Direct Dilution Method

Objectives:

When the participants complete this topic they should be able to:

1. Identify the proper apparatus and chemicals needed to set up BODs by the direct dilution method.
2. Conduct a BOD test (direct dilution method with DO meter) given proper test equipment, procedures sheet and sample.
3. Translate the raw data from the BOD test into proper units of expression given appropriate equations.

Instructional Aids:

Handout
Lab equipment

Instructional Approach:

Lecture
Lab

References:

Standard Methods

Class Assignments:

Module No:	Topic: Direct Dilution Method

Instructor Notes:	Instructor Outline:
	1. List and identify apparatus and chemicals needed to set up BODs using a DO meter.
Pages 36 - 43	2. a. Demonstrate the setup of the test.
	b. Have participants set up the BOD test.
	3. Give participants data from a five day BOD test and have them calculate the BODs.

BIOCHEMICAL OXYGEN DEMAND (BOD)

Using DO Meter with BOD Probe

Introduction

The biochemical oxygen demand (BOD) is defined as the quantity of oxygen used in the biochemical oxidation of organic matter in a specified time, at a specified temperature, and under specific conditions. The standard BOD test performed on domestic waste is carried out for 5 days at 20 C. The BOD test is used as a measure of the organic strength of sewage. If the sewage is strong, for example, it will contain a large amount of decomposable organic material. In such a case, the oxygen requirement and BOD would be large. By the same argument, sewage containing small amounts of decomposable organic materials would have a small BOD.

The test is performed by determining the amount of dissolved oxygen in the sample at the start and comparing it to the amount of dissolved oxygen in the sample after five days. The dissolved oxygen depleted over this period has been used to stabilize organic material and is therefore the biochemical oxygen demand (BOD) of the sample.

Whenever chlorinated samples are collected for the determination of BOD, sufficient reducing agent must be added to the sample to destroy the chlorine. After dechlorination, the sample must be "reseeded" with organisms.

Equipment

300 ml BOD bottles with ground glass stoppers

Incubator 20 C.

Pipettes - 10 ml. graduated, large tip base

Siphon apparatus for dilution water

Three 5 ml. measuring pipettes with bulbs (or three 2 ml automatic pipettes)

500 ml. wide-mouth Erlenmeyer flasks

50 ml. buret, with 0.1 ml. graduations

BOD bottle air-trap caps

DO meter with BOD probe

Reagents

1. CALCIUM CHLORIDE SOLUTION

   Dissolve 27.5 g. anhydrous calcium chloride ($CaCl_2$) in 500-600 ml. distilled water and make up to one liter with distilled water.

2. MAGNESIUM SULFATE SOLUTION

   Dissolve 22.5 g. magnesium sulfate ($MgSO_4.7H_2O$) in 500-600 ml. distilled water and make up to one liter with distilled water.

3. IRON (III) CHLORIDE (FERRIC CHLORIDE) SOLUTION

   Dissolve 0.25 Iron (III) chloride ($FeCl_3.6H_2O$) in 500-600 ml. distilled water and make up to one liter with distilled water.

4. PHOSPHATE BUFFER SOLUTION

   Dissolve 8.5 g. monobasic potassium phosphate (KH_2PO_4), 21.75 g. dibasic potassium phosphate (K_2HPO_4), 33.4 dibasic sodium phosphate ($Na_2HPO_4.7H_2O$) and 1.7 g. ammonium chloride (NH_4Cl) in 500-600 ml. distilled water. After salts have dissolved, make up to one liter with distilled water. The pH of this buffer should be about 7.2 and should be checked.

5. DILUTION WATER

The dilution water is made by adding 1 ml. each of solution #1 (Calcium chloride), #2 (Magnesium sulfate) and #3 (Ferric chloride) in that order to each liter of distilled water. This solution is then aerated by shaking partially filled bottles or by bubbling clean compressed air through the liquid for 24 hours. The solution should then age at least 24 hours. Just before use, add 1 ml. of solution #4 (Phosphate buffer) to each liter of distilled water.

Procedure for Non-chlorinated Samples

1. SET UP SEVEN BOD BOTTLES

Duplicates of three different dilutions of the sample should be run determining initial DO on each dilution and final DO of each dilution. In addition initial DO should be determined on the dilution water, and final DO on the dilution water. The same bottles are used for initial and final DO determinations.

Initial DO
&
5-Day DO
(Final)

1st 2nd 3rd
Three Dilutions

Dilution
Water

Diagram of BOD bottle arrangement

2. SET UP SAMPLE DILUTIONS

Samples containing residual chlorine should be dechlorinated by adding sodium sulfite. BOD dilutions can be prepared with seeded standard dilution water. For procedures for seeding and dechlorination refer to "Standard Methods", 13th edition.

Use Table I below to determine proper dilutions. Make dilution directly into the 300 ml. BOD bottles. For example, if you anticipate a BOD of 30 ppm you should make dilutions using 30 ml., 37.5 ml ~~and~~ 60 ml of sample. Pipette 30 ml. into the three bottles used for the first dilution, 37.5 ml. into the three second-dilution bottles, and 60 ml. into the three third-dilution bottles. Then fill each bottle with dilution water using the siphon apparatus to avoid excessive aeration of sample. Replace stopper, being sure not to trap air and mix by inverting several times.

TABLE I
(BOD test Range)

Dilution Factor	Ml of Sample Needed/300 ml.	Anticipated BOD Range, ppm
1	300	2 - 5
2	150	4 - 10
4	75	8 - 20
5	60	10 - 25
8	37.5	16 - 40
10	30	20 - 50
20	15	40 - 100
25	12	50 - 125
40	7.5	80 - 200
50	6	100 - 250
66.7	4.5	133 - 333
100	3	200 - 500
300	1.5	400 - 1000
400	0.75	800 - 2000

3. RUN INITIAL DO

Run DO on the bottles for each dilution and dilution water using the DO meter.

4. PLACE FINAL DO BOTTLE IN INCUBATION

Incubate for 5 days (± 2 hours) at 20 C. Make air trap by putting a couple ml. distilled water around base of stopper and covering with special BOD bottle caps.

5. RUN FINAL DO

After 5 days on the dilution water blanks and on samples.

Five day BOD sample = (Initial DO of the sample - Final DO of sample) X dilution factor.

Example:

Initial DO of sample	= 8.0 mg/l
Final DO of sample	= 4.0 mg/l
ml. of sample	= 30 ml.
Initial DO of dilution water	= 8.0 mg/l
Final DO of dilution water	= 7.7 mg/l
5 day BOD of sample	= (8.0 - 4.0) x 10
	= 4 x 10
	= 40 mg/l

Notes:

To find dilution factor for each sample refer back to column I of Table I.

The oxygen depletion due to the dilution water is not taken into the calculations of BOD. This is used only as a check on the dilution water. If the depletion ~~is greater~~ than 0.3 mg/l for the dilution water, the data cannot be considered valid. New dilution water should be prepared and the tests re-run.

BOD Chlorinated Samples

1. SECURE AN UNCHLORINATED SAMPLE OF RAW SEWAGE

Secure an unchlorinated sample of raw sewage or primary effluent 24 hours prior to the time the BOD test is to be set-up. Collect about 1 liter of unchlorinated sample and let stand at room temperature overnight. Pour off the clear portion of the sample and use it for the "Seed".

2. PRESENCE OF CHLORINE

Check for the presence of chlorine in the sample to be evaluated as follows:

a. Carefully measure 100 ml. of well-mixed sample into a 250 ml. Erlenmeyer flask.

b. Add a few crystals of potassium iodide (KI) to the sample and dissolve the crystals.

c. Add 1 ml. of concentrated sulfuric acid (H_2SO_4) and mix well.

d. Add ~~five~~ drops of starch. If no blue color is produced then chlorine is absent, the BOD of the sample may be determined without further treatment.

If a blue color is produced, titrate the sample using 0.025 N. sodium thiosulfate ($Na_2S_2O_3$) to the endpoint between the last trace of blue color and a colorless solution (colorless endpoint). Make the titration very slowly, counting the drops of "thio" used and record this number.

3. DECHLORINATING A SAMPLE

To dechlorinate a sample, measure out 100 mls. of well-mixed sample into a clean 250 ml. Erlenmeyer flask. Add the number of drops of "thio" determined necessary for dechlorination. (Step #2 above). Mix well. Use this sample for determination of BOD. If more sample is needed place a larger sample (measure carefully) into a clean container and add a proportionate number of drops of the "thio" for dechlorination.

4. SEEDING OF THE SAMPLE

For seeding of the sample, add 1 ml. of the aged seed (Step #1 above) to each of the BOD bottles containing dechlorinated sample. Also set up samples of the seed for determination of the BOD using 2, 3, and 4 percent (6, 9, and 12 mls. seed) and determine the 5-day depletion due to 1 ml. of seed.

Calculations

$$\frac{\text{5-day DO depletion of seed sample}}{\text{mls. of seed}} = \text{mg/l DO Depletion caused by each ml. of seed}$$

Initial DO (mg/l) of diluted sample - (final DO + seed correction factor) x dilution factor = BOD

Or

IDO - (FDO + SCF) x DF = BOD mg/l

B.O.D. WORKSHEET

SAMPLE SOURCE	% DIL.	DATE IN	DATE OUT	INITIAL D.O.	FINAL D.O. 1	2	AVE.	DIFF.	mg/l BOD

FORMULA $\frac{\text{DIFF.}}{\text{\% DIL.}} \times 100 = \text{mg/l BOD}$

Module No:	Module Title:
	Submodule Title: BOD
Approx. Time: 30 Min.	Topic: Summary of Methods

Objectives:

When the participants complete this topic they should be able to:

1. Differentiate between the three BOD test methods as covered in topics by comparing the following parameters:

   a. Time required to complete the test
   b. Equipment and reagents required
   c. Precision and accuracy
   d. Interferences encountered

2. Recommend a method using the above parameters for use in "home" plant.

Instructional Aids:

Handouts

Instructional Approach:

Discussion

References:

Standard Methods
EPA EMPs Manual

Class Assignments:

Module No:	Topic: Summary of Methods

Instructor Notes:	Instructor Outline:
	Compare and contrast the two methods.